[Jpn. J. Parasitol., Vol. 35, No. 6, 475–482, December, 1986]

Experimental Chagas' Disease in Athymic Nude (rnu/rnu) Rats: Histopathological Characteristics and Kinetics of Appearance of Amastigotes

SACHIO MIURA AND TSUTOMU TAKEUCHI

(Received for publication; January 21, 1986)

Key words: *Trypanosoma cruzi*, protozoa, parasitic, Chagas' disease, natural resistance, myocarditis

Introduction

Investigations on the cellular mechanism which controls susceptibility of hosts to parasites are important for developing the protective measures against parasitic diseases. Such studies on Chagas' disease caused by a parasitic protozoon, *Trypanosoma cruzi*, have been conducted on mice and partially on rats. For instance, previous data on these animal models indicated that administration of anti-thymocyte serum in mice (Roberson *et al.*, 1973b), neonatal thymectomy in mice (Behbehani, 1971) and in rats (Roberson *et al.*, 1973b), and the use of athymic nude (nu/nu) mice (Kierszenbaum and Pienkowski, 1979) increased the susceptibility to *T. cruzi* infection. Other treatments to depress the thymus-related immunological activities also showed their importance in the controlling mechanism (Roberson *et al.*, 1973b; Kierszenbaum and Howard, 1976; Rodriguez *et al.*, 1981). However, differences in the susceptibility between the control and the experimental with the depressed immunological activities demonstrated in these previous models did not seem necessarily sufficient for further studies on the controlling mechanism, because mice, employed in some of these studies, were usually susceptible to *T. cruzi* infection even if their immunological activities were not depressed (Kierszenbaum and Pienkowski, 1979), and the parasitemia in thymectomized rats became negligible during the course of infection (Roberson *et al.*, 1973b). Experimental models with more conspicuous differences in the susceptibility to this parasitic infection between the control and the experimental, therefore, appear to be useful.

Among the experimental models recently tested, athymic nude (rnu/rnu) rats exhibited much higher susceptibility to *T. cruzi* infection than the heterozygous, thymus-bearing littermates (rnu/+) (Miura *et al.*, 1982; Rodriguez *et al.*, 1983). Accordingly, the experimental infection of these rats with *T. cruzi* seemed to be worth further evaluation. The present study was attempted, because basic histopathological studies, although such investigations are evidently useful for characterization of the cellular mechanism controlling the susceptibility of hosts to *T. cruzi* infection, have not been done on the present experimental model.

Materials and Methods

Tulahuen strain of *T. cruzi*, which had been maintained in BALB/c mice by intraperitoneal inoculation of the blood, was used in this study. Sprague-Dawley athymic nude (rnu/rnu) rats and the heterozygous, thymus-bearing littermates (rnu/+), both of which were males of 5–9 weeks of age, were supplied by Kitasato Biomedical Laboratory (Tokyo, Japan). Two to

Department of Parasitology, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160, Japan.

three rats were maintained in a cage placed in a clean laminar-flow rack (Ishihara Co. Ltd., Tokyo, Japan). The following experiments were done sterilely when necessary.

Trypomastigotes (blood froms) of *T. cruzi* obtained on the 10th day of infection from BALB/c mice by cardiac puncture in the presence of 0.05% heparin were washed once and concentrated by centrifugation. Approximately 1.5 x 10^6 trypomastigotes, suspended in 0.5 ml physiological saline, were inoculated into each of six nude rats and the same number of littermates. Subsequently, the parasitemia was evaluated on the blood obtained from the cervical vein at appropriate time intervals using a haemocytometer. Counting was done three times on each blood specimen, and the average number of *T. cruzi* in these rats was calculated.

To investigate the histopathological characteristics of the present experimental model, nude rats and the littermates infected as above were sacrificed by rapidly extracting the whole blood through cardiac puncture under a light anesthesia, and histopathological slides of the several tissues were immediately prepared in the conventional manners. To compare with the data on nude rats, BALB/c mice (male, 5–6 weeks of age) were infected with 10^5 of *T. cruzi* blood forms and processed as above.

The kinetics of appearance of *T. cruzi* amastigotes was examined by inoculating the tissue extracts of nude rats and the littermates,

Table 1 Kinetics of appearance of *Trypanosoma cruzi* amastigotes in the tissues of nude (rnu/rnu) rats and the littermates (rnu/+)

Tissue extracts inoculated into mice	Weeks after infection of nude rats or the littermates with *T. cruzi*			
	1	2	3	14
	No. of *T. cruzi* in the blood of the inoculated mice / 100 light microscopic fields			
Nude rats				
Heart muscle	57±29	2092±680	7063±63	
Skeletal muscle	25±15	382±246	8800±2520	
Large intestine	4±4	224±200	14165±1830	
Liver	1731±155	1190±864	8060±1830	
Spleen	2630±1600	1029±141	16364±3600	
Kidney	2231±200	731±432	10333±333	
Littermates				
Heart muscle		0	42±31	0
Skeletal muscle		254±20	6460±1470	0
Large intestine		0	0	0
Liver		376±76	233±96	0
Spleen		209±88	178±100	0
Kidney		31±19	254±199	0

Six nude rats and an equal number of littermates were infected with *T. cruzi*. Two of the infected nude rats were sacrificed on the 1st, 2nd and 3rd week. The same number of littermates were sacrificed on the 2nd, 3rd and 14th week. The numerical values represent averages ± standard deviations calculated from the number of *T. cruzi* trypomastigotes in the eight mice inoculated with each tissue extract of these infected rats. The experiments were done twice on C3H mice and once on DDY mice. There was no significant difference in the number of *T. cruzi* blood forms between these two strains of mice.

which were infected and sacrificed as described above, into mice. The tissues of these infected rats were removed, weighed, rinsed and homogenized in physiological saline to yield 0.2 g wet weight/ml. One-half ml of each tissue extract was inoculated intraperitoneally into each of four C3H/HeN or DDY mice (male, 5–6 weeks of age). In 14 days, the total number of trypomastigotes in 100 different light microscopic fields was counted on 0.025 ml of the blood, which was obtained by ocular puncture from each of the inoculated mice and placed on a 25 x 75 mm slide glass with a 18 x 24 mm cover glass, under a magnification of x600. Counting was repeated three times on each of the specimens. Although the kinetics was examined by directly counting the number of amastigotes on the stained histopathological slides as previous investigators conducted (Roberson *et al.*, 1973b), the number of *T. cruzi* in the tissues of nude rats was frequently too large to be counted, particularly in the right ventricle (see Fig. 6). Moreover, this procedure could not be applicable to the littermates because of few *T. cruzi* in the tissues (see Fig. 2 and Table 1). The usefulness of the present method was confirmed by our preliminary study that the intensity of parasitemia of C3H and DDY mice infected with Tulahuen strain of *T. cruzi* was well in accord with the size of initial inoculum (Miura *et al.*, unpublished observation).

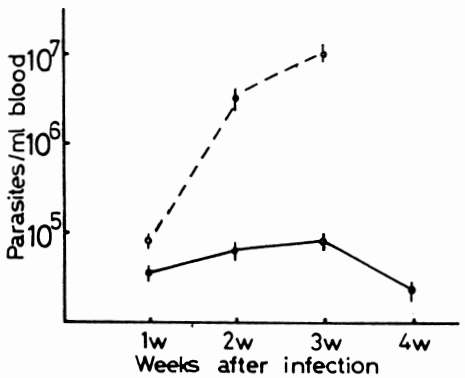
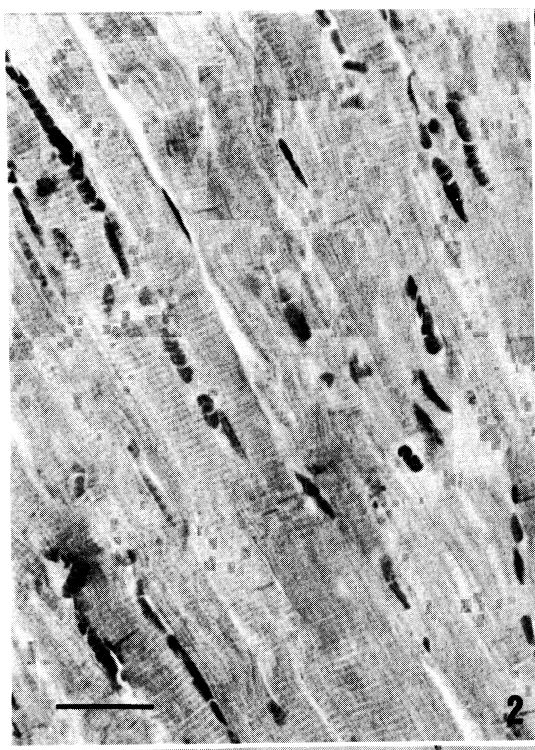


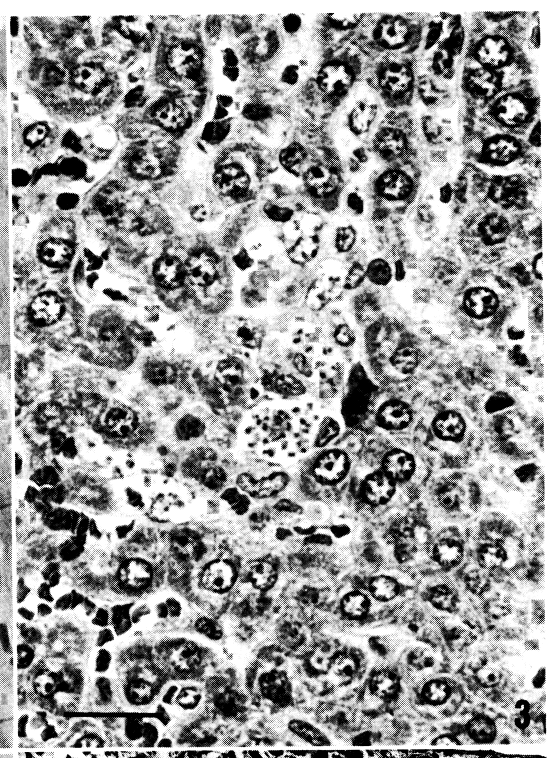
Fig. 1 Parasitemia in nude (rnu/rnu) rats (dotted line) and in the littermates (rnu/+) (solid line) infected with Tulahuen strain of *Trypanosoma cruzi*. The data stand for averages ± standard deviations calculated from three independent determinations.

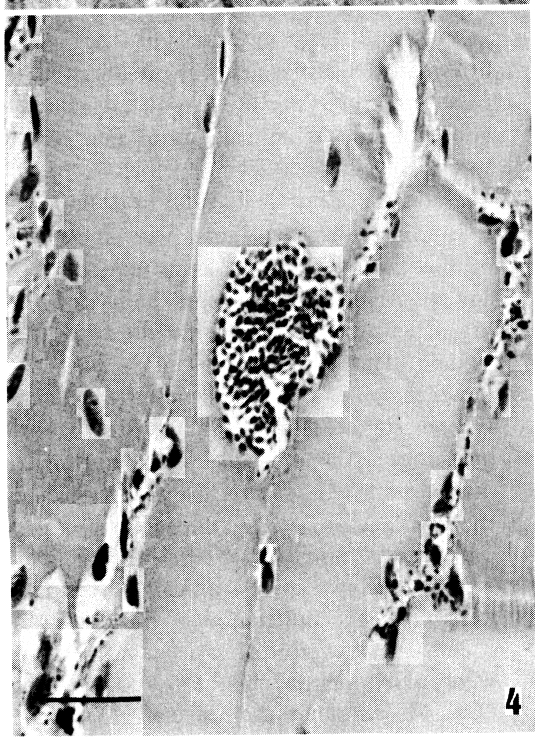
Results

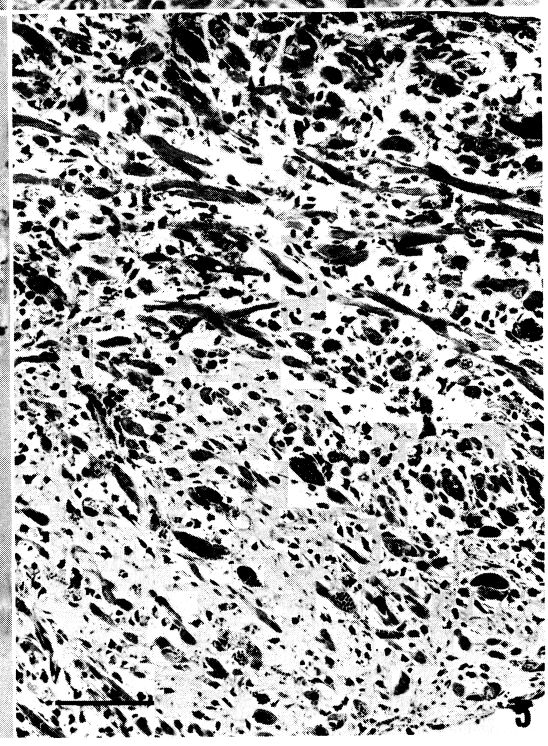
The parasitemia in these infected rats was shown in Fig. 1. The infected nude rats exhibited the intensive parasitemia and died in 3 weeks, whereas the littermates survived as long as the observation was made, i.e., for longer than 6 months. The parasitemia in the littermates was significantly lower than that in nude rats from the 1st to 3rd week of infection; moreover, it became negligible after the 5th week, which was confirmed by the absence of epimastigotes in LIT and NNN media inoculated with 0.5 ml of the blood and observed for two months (data not shown).

Light microscopically, the amastigotes were barely detectable in the heart muscle (Fig. 2), skeletal muscle, brain, lung, liver, spleen, kidney and large intestine of the infected littermates. Significant histopathological lesions were not observed throughout the course of infection in this animal group. For instance, the heart muscle suffered neither histopathological damage nor cellular infiltration (Fig. 2). In contrast, the amastigotes were found in all of the tissues of nude rats examined (Figs. 3 and 4). Among these tissues, the heart muscle, in particular the muscle of right atrium (Fig. 5) and right ventricle (Fig. 6) exhibited the presence of numerous, large pseudocysts of the amastigotes as well as the intensive histopathological damage such as interruption and disappearance of the muscle fibers caused by the protozoa. Despite the presence of the pseudocysts, tissues other than the heart muscle did not show any significant degeneration except for the infiltration of host cells (Figs. 3 and 4). Myocardiac lesions of BALB/c mice were much lighter than those of nude rats (Fig. 7), although the infected mice usually died in 10–14 days after infection. The only significant histopathological finding in the


2


3


4


5

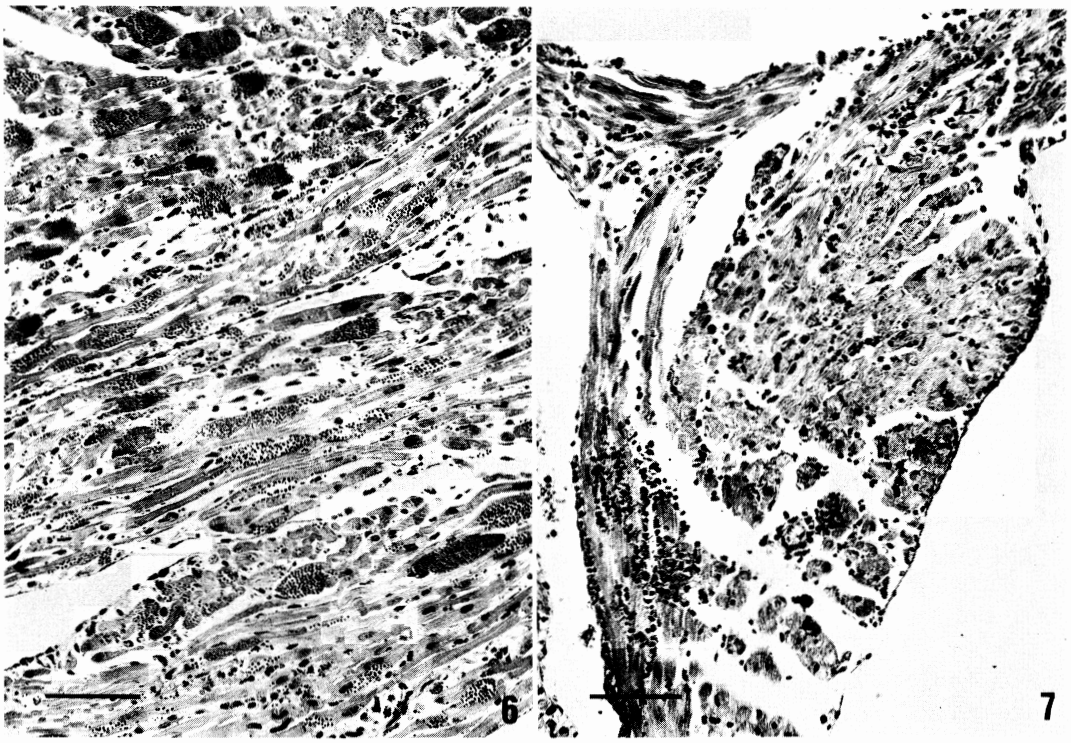


Fig. 6 Right ventricle of the infected nude rats on the 2nd week. Note numerous, large pseudocysts containing *T. cruzi* amastigotes. Degeneration of the muscle fibers is evident. Bar = 40 μm.

Fig. 7 Right atrium of BALB/c mice not long before the death, i.e., on the 10th day of infection with *T. cruzi*. Significant histopathological damages are not found except for the infiltration of host cells. Bar = 40 μm.

heart muscle seemed to be the infiltration of host cells. The number of pseudocysts and of amastigotes per one pseudocyst in the heart muscle of BALB/c mice were smaller than those of nude rats.

The kinetics of appearance of *T. cruzi* amastigotes was summarized in Table 1. The amastigotes seemed to appear mainly in the skeletal muscle of the littermates on the 3rd week of infection without any significant histopathological damage. No trypomastigotes were found, however, in any of the mice inoculated with each tissue extract of the littermates on the 14th week. On the other hand, the intensive parasitemia was observed in most of the mice inoculated with each of the tissue extracts of nude rats; particularly in those with the extract of liver, spleen or kidney on the 1st week, and with any of the tissue extracts on the 3rd week. The number

Fig. 2 Muscle of the right ventricle of the littermates on the 3rd week of infection with *T. cruzi*. Significant histopathological lesions are not found. The slides demonstrated in Figs. 2 to 7 were stained with hematoxylin-eosin. Bar = 10 μm.

Fig. 3 Liver of nude rats 2 weeks after the infection with *T. cruzi*. A few pseudocysts of the amastigotes are found; however, significant histopathological damages are absent. Bar = 10 μm.

Fig. 4 Skeletal muscle of the infected nude rats on the 2nd week. A few pseudocysts of *T. cruzi* amastigotes and the infiltration of host cells are demonstrated. Degeneration of the muscle is not observed. Bar = 10 μm.

Fig. 5 Right atrium of the infected nude rats on the 2nd week. The muscle fibers are extensively degenerated. Bar = 40 μm.

of amastigotes in the heart muscle of nude rats seemed to be considerably small at first, but rapidly increased during the 1st and 2nd week.

Discussion

The present findings suggest that nude rats are highly susceptible to *T. cruzi* infection, while the littermates are not. This appears compatible with the data obtained by previous studies which utilized mice and rats with the depressed thymus-related immunological activities (Behbehani, 1971; Roberson *et al.*, 1973a; 1973b; Kierszenbaum and Howard, 1976; Kierszenbaum and Pienkowski, 1979; Rodriguez *et al.*, 1981). However, the observations on such aspects as the intensity of parasitemia and the survival period suggest that the difference in the susceptibility to *T. cruzi* infection between nude rats and the littermates is more conspicuous than that between the control and the experimental in the previous models, although strains of *T. cruzi* employed were not always the same.

Our histopathological investigations suggest that *T. cruzi* infection in nude rats is characterized by the intensive myocardiac lesions, particularly those in the right atrium and right ventricle. Such myocardiac lesions were not found in BALB/c mice, in spite of the fact that the mice infected with *T. cruzi* died in 10–14 days. The littermates also lacked the myocardiac damage and *T. cruzi* appeared to multiply primarily in the skeletal muscle but not in the heart muscle. Although Mercado (1976) indicated that Tulahuen strain of *T. cruzi* was reticulotropic in mice, our data suggest that this strain appeared myotropic in rats. The absence of evident histopathological lesions from the liver of nude rats as well as of the littermates seems to support this view. Moreover, the present findings may indicate that the tissue affinity of *T. cruzi* in nude rats is different from that in the littermates. We envision, at present, that the changed affinity may be attributed to T-cell deficiency in nude rats. It also seems possible that the natural heterogeneity in a strain of *T. cruzi* (Dvorak, 1984) in combination with T-cell deficiency is responsible, at least partially, for the changed affinity. Although similar increased myocardiac damages by *T. cruzi* have been reported by Kumar *et al.* (1970), the strain employed in their study was originally myotropic.

Concerning the intensive myocardiac damage in nude rats, the rapid increment in the number of *T. cruzi* in the heart muscle during the 1st and 2nd week, as demonstrated in Table 1, may be responsible for its development. However, the evident increase in the number of *T. cruzi* amastigotes in the skeletal muscle and large intestine of nude rats during the 2nd and 3rd week does not seem to affect the development of lesions in these tissues. The reason of this difference is not known at present.

These data led us to conceive that nude rats offer an interesting model for screening the drugs against myocardiac lesions in Chagas' disease as well as for examining the mechanism of natural resistance against this parasitic infection. In particular, the role of thymus function in the cellular mechanism which controls the susceptibility to *T. cruzi* infection and determines the tissue affinity of this parasite may be effectively investigated using the present experimental model.

Summary

The susceptibility of athymic nude (rnu/rnu) rats to *Trypanosoma cruzi* infection was investigated. The infected nude rats showed the intensive parasitemia and died in 3 weeks, whereas the parasitemia in the littermates (rnu/+) was low at first and negligible after the 5th week. The infected littermates survived for at least 6 months. *T. cruzi* was found in all of the tissues of nude rats examined histopathologically; in particular, a large number of amastigotes were detected in the right atrium and right ventricle which also exhibited the intensive histopathological lesions. In

contrast, such histopathological damages were scarcely found in the littermates. The mice inoculated with the extract of either liver, spleen or kidney of the nude rats sacrificed on the 1st week after infection showed the higher parasitemia than those with other tissue extracts, while the rate of increase in the number of *T. cruzi* during the 1st and 2nd week seemed rapid in the heart muscle. Among the mice inoculated with the tissue extracts of the littermates on the 3rd week, only those with the extract of skeletal muscle indicated the considerably high parasitemia; however, no *T. cruzi* was demonstrated in any of the mice inoculated on the 14th week. These data suggest that there is a marked difference in the susceptibility to *T. cruzi* infection between nude rats and the littermates.

References

1) Behbehani, M. K. (1971): *Trypanosoma* (*Schizotrypanum*) *cruzi* infection in X-irradiated and thymectomized mice. Trans. Roy. Soc. Trop. Med. Hyg., 65, 265.
2) Dvorak, J. A. (1984): The natural heterogeneity of *Trypanosoma cruzi*: Biological and medical implications. J. Cell Biochem., 24, 357–371.
3) Federici, E. E., Ablemann, W. H. and Neva, F. A. (1964): Chronic and progressive myocarditis and myositis in C3H mice infected with *Trypanosoma cruzi*. Am. J. Trop. Med. Hyg., 13, 272–280.
4) Kierszenbaum, F. and Howard, J. G. (1976): Mechanism of resistance against experimental *Trypanosoma cruzi* infection: The importance of antibodies and antibody forming capacity in the Biozzi high and low responding mice. J. Immunol., 116, 1208–1211.
5) Kierszenbaum, F. and Pienkowski, M. M. (1979): Thymus-dependent control of host defense mechanisms against *Trypanosoma cruzi* infection. Infect. Immun., 24, 117–120.
6) Kumar, R., Kline, I. K. and Ablemann, W. H. (1970): Immunosuppression in experimental acute and subacute Chagasic myocarditis. Am. J. Trop. Med. Hyg., 19, 932–939.
7) Mercado, T. I. (1976): *Trypanosoma cruzi*: Lactate dehydrogenase isoenzymes and infection in mice. Exp. Parasitol., 40, 411–420.
8) Miura, S., Takeuchi, T., Kobayashi, S. and Asami, K. (1982): Mechanism of natural resistance of rats against *Trypanosoma cruzi*. Jpn. J. Parasitol., 31 (supplement), 85 (in Japanese).
9) Roberson, E. L., Chapman, Jr., W.L. and Hanson, W. L. (1973a): The effects of total-body X-irradiation on *Trypanosoma cruzi* infection (Chagas' disease) in mice and rats. Z. Parasitenk., 41, 83–94.
10) Roberson, E. L., Hanson, W. L. and Chapman, Jr., W. L. (1973b): *Trypanosoma cruzi*: Effects of anti-thymocyte serum in mice and neonatal thymectomy in rats. Exp. Parasitol., 34, 168–180.
11) Rodriguez, A. M., Afchain, D., Santoro, F., Bazin, H. and Capron, A. (1983): Parasitological and immunological aspects of *Trypanosoma cruzi* infection in nude rats. Z. Parasitenk., 69, 141–147.
12) Rodriguez, A. M., Santoro, F., Afchain, D., Bazin, H. and Capron, A. (1981): *Trypanosoma cruzi* infection in B-cell-deficient rats. Infect. Immun., 31, 524–529.

先天性胸腺欠損ラット（ヌードラット）における実験シャーガス病に関する研究：組織病理所見と臓器での虫体の動態について

三浦左千夫　竹内　勤
（慶応義塾大学医学部寄生虫学教室）

先天性胸腺欠損ラット（ヌードラット，rnu/rnu）のトリパノソーマ・クルジー（Tulahuen 株）に対する感受性について検索した．その結果，ヌードラット（５～９週令，雄）では感染直後より血中虫体数が急速に増加し，３週間以内に全てのラットが死亡することが判明した．一方，胸腺を有している同系のlittermate（rnu/$_+$）においてはパラシテミアの程度は低く，感染５週目以後血中より原虫が検出されなくなった．これら littermate は少なくとも６ヶ月以上は生存した．ヌードラットにおいては amastigote は検索した全臓器に見出されたが，特に右心室，右心房の筋細胞内に極めて多数検出された．また，これに対応し組織病理学的にも高度の筋の破壊，変性が観察された．他臓器においては虫体は見出されるものの組織病理学的異常所見はそれほど顕著ではなかった．ヌードラット各臓器内の amastigote の動態に関してはまず感染１週目には肝，脾，腎に虫体が集中することが示唆された．しかし，感染１から２週目に至る虫体の増加の割合に関しては心筋が最も高かった．一方，littermate においては感染３週目には骨格筋に虫体が多く見られるものと思われたが，感染14週目に至ると検索したどの臓器からも虫体は消失していた．以上のデータはヌードラットとその littermate の間にはトリパノソーマ・クルジーに対する感受性の点で非常に大きな差異があることを示唆している．本実験系はこの原虫感染に対する宿主の抵抗性のメカニズムを追求する上で有用と考えられた．